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Race influences survival in glioblastoma patients with KPS 80 and associates with genetic markers of retinoic acid metabolism

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Abstract

Purpose: To study whether the clinical outcome and molecular biology of gliomas in African-American patients fundamentally differ from those occurring in Whites.

Methods: The clinical information and molecular profiles (including gene expression array, non-silent somatic mutation, DNA methylation and protein expression) were downloaded from The Cancer Genome Atlas (TCGA). Electronic medical records were abstracted from Northwestern Medicine Enterprise Data Warehouse (NMEDW) for analysis as well. Grade II-IV Glioma patients were all included.

Results: 931 Whites and 64 African-American glioma patients from TCGA were analyzed. African-American with Karnofsky Performance Score (KPS) 80 have significantly lower risk of death than similar White Grade IV Glioblastoma (GBM) patients (HR[95%CI]=0.47[0.23, 0.98], P=0.0444, C-index=0.68). Therefore, we further compared gene expression profiles between African-American GBM patients and Whites with KPS 80. Extrapolation of genes significantly associated with increased African-American patient survival revealed a set of 13 genes with a possible role in this association, including elevated expression of genes previously identified as increased in African-American breast and colon cancer patients (e.g. CRYBB2). Furthermore, gene set enrichment analysis revealed retinoic acid (RA) metabolism as a pathway significantly upregulated in African-American GBM patients who survive longer than Whites (Z-score=-2.10, Adjusted P-value=0.0449).

Conclusions: African Americans have prolonged survival with glioma which is influenced only by initial KPS score. Genes previously associated with both racial disparities in cancer and pathways associated with RA metabolism may play an important role in glioma etiology. In the

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future exploration of these genes and pathways may inform novel therapies for this incurable disease.

Keywords

African Americans; Whites; Glioma; Retinoic Acid Metabolism; Karnofsky Performance Score

Introduction

Gliomas are the most common type of primary brain tumor in adults, which account for 80% of all malignant brain tumors. According to degree of malignancy and pathologic evaluation, gliomas are graded from I to IV according to the WHO classification scheme, in which grade I is consistent with a low-grade pilocytic astrocytoma, and grade II & III are further categorized together [1], whereas GBM, accounting for the majority of gliomas (56.6%), is the most challenging with a five-year survival rate as low as 5% [2]. The incidence rate in Whites is 2 to 20-fold higher compared to African-Americans[3–5]. Because of the relative paucity of African-American patients, most published race-based analyses have been based on the Surveillance, Epidemiology and End Results (SEER) program. However, those analyses could be biased as they did not include the information of KPS, which was recognized as an important factor associated with survival in glioma patients [3–5]. As a result, it is still not clear whether the relatively few gliomas that do arise in African-American patients are fundamentally different from those arising in Whites, either clinically or molecularly.

Recent advances in large scale data collection have greatly facilitated the understanding of the molecular features of gliomas. For example, TCGA provides detailed genomic data, along with clinical and pathologic data, and has been used to demonstrate different genomic landscapes between African-American and White breast cancer patients [6]. However, no genome-wide study has determined what prognostic factors differ between African-American versus White patients with glioma. Herein, we report the results of a rigorous study which analyzed the racial disparity between White and African-American glioma patients from clinical and molecular perspectives, to further understand race-based biological differences in gliomas.

Methods

Study Cohort

TCGA clinical and level 3 genomic data were downloaded from University of California, Santa Cruz(UCSC) Cancer Genomics Browser in June 7, 2016 (<https://genome-cancer.ucsc.edu/>) for grade II-IV gliomas. Grade II-III patients in this study, as defined in TCGA database, included Astrocytoma, Oligoastrocytoma, and Oligodendroglioma. Race and ethnicity information were abstracted from TCGA Data Portal website(<http://tcga-data.nci.nih.gov/tcga/>). The electronic clinical information of all the glioma patients treated at Northwestern Memorial Hospital (NMH) between 2006 and 2016 were also obtained under IRB–approved protocols from Northwestern Medicine Enterprise Data Warehouse

(NMEDW). Insufficient genomic data was available for NMH patients. Data were all de-identified, processed and prepared as described in the supplementary file.

Descriptive analysis

The clinical characteristics of the patients were described as mean (SD) for continuous variables, and number (percentage) for categorical variables. For continuous variables, differences between African-American and White patients were analyzed by Student's t-test or Wilcoxon rank sum test as appropriate, while Chi-square or Fisher's exact test were used for categorical variables.

Survival analysis

Cox proportional hazards model was carried out to evaluate the relationship between race and overall survival. Backwards stepdown selection was used to arrive at a set of factors to be adjusted in the model, with bootstrap metrics to penalize predictive ability for variable selection [7]. Bootstrap and predictive mean matching (PMM) with 1000 imputations for each dataset were conducted for missing data imputation [8,9]. A restricted cubic spline was used to model the nonlinear relationship between age and overall survival in the Cox model. The number of knots that generated the smallest Akaike's information criterion (AIC) was adopted for modeling the spline of age. The interaction between race and the other variables was considered, but only statistically significant interactions were included in the final model. The final model was validated for Somers' Dxy rank correlation between predicted log hazard and observed survival time. The concordance index (C index) for each model was then calculated by $\frac{D_{xy} + 1}{2}$ [10]. The bootstrap was used (with 300 resamples) to penalize for possible overfitting. Subgroup analysis as well as sensitivity analysis were conducted when there was an interaction. Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). All the tests were two-sided and $P < 0.05$ was considered significant unless specified. For all the subgroup analyses, false discovery rates (FDRs) by Benjamini-Hochberg were used for multiple correction, with 0.10 as the threshold of significance.

Gene expression analysis

The Level 3 gene expression profile measured by Affymetrix HT Human Genome U133a microarray platform were used for analysis. The imputed clinical data was combined with gene expression for each patient. Differences in the selected clinical factors between African-Americans and White patients in this dataset were first checked to see whether any clinical factor needed to be adjusted. The difference of expression of all the genes between the two groups were examined by Wilcoxon rank sum test when the two groups were balanced on all the factors. Otherwise, linear regression was conducted with adjustment of the confounding factors (factors that were statistically significant different between two races). Differentially expressed genes were filtered out at fold change 1.5 and false discovery rates (FDRs) by Benjamini-Hochberg < 0.10 . To evaluate the prognostic effectiveness of the selected genes, we developed a risk-score formula for predicting survival. The risk score for each patient was a linear combination of the mRNA expression level weighted by the regression coefficient derived from the univariate Cox regression

analysis [11]. We next divided patients into high-risk and low-risk groups using recursive partitioning analysis to determine the optimal cut-off point for predicting survival [12]. Then survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. Cox proportional hazards regression analysis was performed to assess the independent contribution of the mRNA signature to survival prediction with the selected adjusted clinical factors.

Unsupervised hierarchical clustering were then conducted to identify clusters of those genes with “euclidean” as the distance measure and “complete” as the clustering method. Brunet algorithm was used to estimate the non-negative matrix factorization [13]. We performed 40 runs for each value of the factorization rank r in range 2:7 to build a consensus map. The optimal clusters were determined by the observed cophenetic correlation between clusters, and validated by silhouette plot and principal component analysis (PCA).

Furthermore, the enrichment of the selected genes on transcription, pathways, ontologies, diseases/drugs, cell types and miscellaneous were analyzed using Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>). The enriched terms were selected at Adjusted P-value <0.05. The gene sets from The Molecular Signatures Database (MSigDB) were used to evaluate their enrichment in the mRNA expression between two race groups using Gene Set Enrichment Analysis v2 software (www.broadinstitute.org/gsea). All the other parameters were set based on their default values.

Results

KPS score interacts with race and survival in glioma patients

A total of 993 glioma patients were included in the analysis. Among them, 929 (93.6%) were Whites and 64 (6.5%) were African-Americans. Their characteristics are summarized in Table 1. African-Americans were more likely to present with a KPS score below 80 (African-Americans 25.0%, Whites 13.7%, $P=0.009$). GBM accounted for 76.56% of African-American patients, which was much higher than the proportions in White patients (76.6% versus 54.1%, $P=0.007$). Otherwise, African-American and White patients were similar in average age, sex, GCIMP status, person neoplasm cancer status, history of neoadjuvant treatment, targeted molecular therapy, radiation therapy and ethnicity (Table 1).

With backward stepdown methods, gender and ethnicity was removed from the Cox regression model for all glioma patients, reducing AIC by 1.07 and 0.73 respectively. There was a nonlinear relationship between age and the log relative hazard among all grade II-IV gliomas ($P=0.006$; Fig.S1A), thus a restricted cubic spline with age of 3 knots was applied; this highlighted a significant interaction between race and KPS ($\chi^2=4.5$, $P=0.033$; Fig. S1B). In the subgroup analysis on KPS, African-American glioma patients had a lower risk of death compared with White patients when KPS ≥ 80 (HR [95%CI] = 0.51 [0.32, 0.89], $P=0.045$, FDR=0.089, C-index=0.81; Fig. 1A & Fig.S1C) compared to KPS<80 ($\chi^2=2.6$, $P=0.10$, FDR=0.115; Fig.S1D). When KPS ≥ 80 , the predicted one-and two-year survival for African-American glioma patients was higher than White patients (Fig.1B–C). An interaction effect between KPS and race in overall survival was also found in the GBM subset ($\chi^2=5.5$, $P=0.019$, Fig.S2B). but not Grade II & III patients ($\chi^2=0.01$, $P=0.92$,

Fig.S2A) African-American GBM patients also showed a better survival than White patients when KPS ≥ 80 (HR [95%CI] = 0.44 [0.21, 0.90], $P=0.025$, FDR=0.089, C-index=0.67; Fig. 1D) but not when KPS<80 ($\chi^2=2.5$, $P=0.11$, FDR=0.089, Fig.S2C). As was observed among all gliomas, the predicted survival of African-American GBM patients was higher than White patients at both one and two years after diagnosis (Fig.1E–F).

The above results were generated with missing data imputed. A sensitivity analysis was conducted using non-imputed data, which showed that there were trends for the interaction between race and KPS for all glioma patients ($P=0.05$) and GBM patients ($P=0.038$). The high absence of reported KPS as well as other variables dramatically reduced the effective sample size, as only complete data will be used for analysis when doing cox regression. So we did not follow with subgroup analysis using KPS as a subgroup factor in the non-imputed data.

However, we did another sensitivity analysis to further investigate the interaction between race and KPS with survival in GBM patients by including more clinical variables in the model: histological type, gene expression subtype, person neoplasm cancer status, initial pathologic diagnosis method. There was also a nonlinear relationship between age and the log relative hazard ($P=0.0054$; Fig.S3A). The interaction between race and KPS remained statistically significant ($\chi^2=4.3$, $P=0.039$; Fig. S3B), and African-American patients still had better survival compared with White patients when KPS ≥ 80 (HR [95%CI] =0.47[0.23, 0.98], $P=0.044$, C-index=0.68; Fig. S3D–E), but not when KPS<80 ($\chi^2=2.1$, $P=0.15$; Fig. S3C). The predicted survival of African-American GBM patients with high KPS was also higher than it was in White patients (Fig. S3F–G).

After the interaction between race and KPS with survival was confirmed by adjusting for additional clinical factors in the TCGA cohort, we conducted another sensitivity analysis to further examine these results by adding glioma patients from NMEDW. There were 929 White and 81 African-American patients in the combined cohort. Even after adjusting for cohort differences, a significant interaction between race and KPS still existed ($\chi^2=4.0$, $P=0.046$; Fig.S4A), and also for hazard ratio and predicted survival (African-Americans vs. Whites: HR [95%CI] =0.50[0.29, 0.85], $P=0.011$; Fig.S4C–E).

Molecular differences between African-American and White GBMs

To discover potential molecular explanations for the increased survival in African-American GBM patients with KPS ≥ 80 , we further analyzed the mRNA expression profiles of TCGA GBM patients whose KPS ≥ 80 . Expression profiles were available for 353 White GBM patients and 16 African-Americans. Because there were a low number of molecular profiles available in African-American patients, we increased our type I error rate to 0.10. All the results on molecular analysis were considered statistically different at P value or FDR<0.10. There was no statistically significant difference between the two groups in any of the evaluated clinical factors (Table S4). However, African-American patients in this imputed dataset still had longer median survival than Whites ($P=0.057$; Fig.2A). We then screened for differentially-expressed genes using Wilcoxon rank sum testing, identifying 13 genes that were differentially expressed between African-American and White GBMs with fold

change 1.5 (Table 2). The risk score for each patient based on these 13 genes was then calculated according to the following formula:

$$\text{Risk score} = -0.06373PRAME + 0.04902CRYBB2 + 0.12008CYP26B1 + 0.05224P2RX5 + 0.10863DKK2 + 0.07206NPY5R - 0.30379TEX14 + 0.10082PTHR1 + 0.01785CAPN6 - 0.20393PTGFR - 0.04084KCNMB3 + 0.29207KERA + 0.13041OCA2$$

Using recursive partitioning analysis, we divided the patients into high-risk and low-risk groups according to risk score at the cut-off of 1.44. The high-risk group showed worse survival compared to the low-risk group ($P < 0.0001$; Fig. 2B). After adjusting for the clinical factors mentioned above, the high-risk group had a 34% higher risk of death than the low-risk group (HR [95% CI] = 1.34[1.01, 1.78], $P = 0.0422$, C-index = 0.87).

Enrichment analysis on KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways found that these 13 genes enriched at Neuroactive ligand-receptor interaction *Homo sapiens* hsa04080 (Adjusted $P = 0.0060$), calcium signaling pathway *Homo sapiens* hsa04020 (Adjusted $P = 0.027$). Enrichment analysis on Gene Ontology (GO) biological process found that these 13 genes enriched at male meiosis (Adjusted $P = 0.024$), negative regulation of retinoic acid receptor signaling pathway (GO: 0048387, Adjusted $P = 0.025$) and regulation of retinoic acid receptor signaling pathway (GO: 0048385, Adjusted $P = 0.025$) (Table 3).

Next, unsupervised hierarchical clustering on the 13 genes was conducted, and two clusters were identified with the highest cophenetic coefficient at 0.98 and an average silhouette width at 0.28 (Fig. S5A–E). Cluster 1 genes includes *PTHR1*, *CYP26B1*, *P2RX5*, *OCA2*, *KCNMB3*, *CRYBB2* and *PRAME*, while in cluster 2 included *DKK2*, *NPY5R*, *TEX14*, *CAPN6*, *PTGFR* and *KERA*. Risk scores based on these two clusters of genes were all calculated for each patient separately. The cut-off value in predicting survival was 1 for cluster 1 genes, and 0.096 for cluster 2 genes, using recursive partitioning analysis. For both clusters of genes, the high-risk group showed worse survival compared to the low-risk group (Cluster 1: $P = 0.025$; Cluster 2: $P < 0.0001$; Fig. S5F).

Discussion

While it is well known that African-Americans are disproportionately at lower risk of glioma than Whites, no studies to our knowledge have addressed whether there also exist race-based clinical and molecular differences among gliomas, and how they relate to patient survival. In the current study we analyzed the interactions of multiple clinical, pathological, and molecular factors in gliomas from African-American and White patients, discovering that African-American GBM patients appear to have longer survival than Whites, but only when KPS is relatively high. Furthermore, pathways associated with RA metabolism may play differing roles in African-American versus White gliomas.

Prior studies have investigated race-based survival differences in glioma patients, with conflicting results. Some have found similar overall survival for African-American and White patients[14], while others reported even better survival outcomes for African-Americans[15] [16]. Hao Xu etc. evaluated 24,262 patients with primary glioblastoma using SEER data, and did not find statistically significance on overall survival between African-

American and White patients, but they did not include KPS in their COX regression model, which was recognized as an important factor associated with survival, thus led to a biased result despite the large sample size.[17] Haley Gittleman etc. built a nomogram to estimate individualized survival probabilities for patients with newly diagnosed GBM, using data from two independent NRG Oncology Radiation Therapy Oncology Group (RTOG) clinical trials. Race was included in the multivariate model but found no association with the survival. However, the number of African-American patients was far too small (13 in the training trial, and 10 in the validation trial) to make any reasonable conclusion on the racial difference as it relates to survival or any meaningful implication.[18] In contrast, one study reported that African-Americans who underwent grossly subtotal resection had worse prognoses compared with similar Whites [19]. In Ostrom et al., survival after diagnosis with GBM among non-Hispanic whites, irrespective of treatment type, was lower than Hispanic whites, blacks, and Asians or Pacific Islanders. These findings are consistent with our own, further substantiating our claim [20].

In our study, we not only included KPS in our model, but also examined the interaction relationship between race and KPS, and considered the non-linear relationship between age and survival as well, which have never seen in other studies on the racial difference. In addition, we conducted three sensitivity analyses to confirm our findings. We found that KPS score is an important clinical factor that differs between White and African Americans, as African-American patients are more likely to have a KPS<80 compared to Whites. The median KPS score of glioma patients overall is approximately 80,[21] and those with a KPS below 70–80 tend to progress faster, which is why this range is normally used as a cut-off in many clinical trials[22]. Our study suggests that although African-Americans have lower incidence of glioma[23], when they are diagnosed, it is more likely to be the most aggressive subtype. The reasons for this are not known, but the lower average socioeconomic status observed among African-Americans may lead to delayed diagnosis and treatment. This is consistent with a previous study on the topic[23], and highlights the difficulty of studying racial factors underlying tumor biology and outcomes.

Adjusting for KPS score therefore allowed us to make more accurate race-based comparisons, determining whether there are any innate biological differences among gliomas by race. When taking KPS, African-American GBM patients survive significantly longer when KPS is ≥ 80 . This interaction was lost in patients with grade II-III gliomas, suggesting that race may play a role only in GBM. However, in our datasets, we could only obtain 15 African-Americans patients with grade II-III gliomas.

From these datasets, we found clusters of race-based gene differences in GBMs that may contribute to enhanced survivorship in African-Americans. One significant discovery is the overexpression of genes related to retinoic acid metabolism. *CYP26B1* and *PRAME*, two of the main genes associated with enhanced survival in African-American patients, both influence retinoic acid signaling. A number of reports have identified the CYP26 enzymes as key mediators that catabolize RA, with evidence suggesting CYP26B1 as a major enzyme that gliomas utilize to break down RA[24,25]. *CYP26B1* is directly related to glioma grade and negatively influences patient survival[25]. Interestingly, *PRAME* is also a dominant repressor of retinoic acid receptor signaling[26], and others have shown that PRAME

overexpression can inhibit the growth of various cancers,[27,28] and has unclear correlations with outcomes in other malignancies[29–31]. PRAME is also highly immunogenic, providing a robust target for immunotherapy [32]. To our knowledge, the specific role that PRAME may have on RA signaling and malignancy in glioma is unknown.

Two key limitations of this study are: (1) it is of limited power from both clinical and molecular perspectives, since the sample size of African-American patients were so limited and hard to magnify. (2) The study's main focus is on TCGA gliomas plus additional data from NMH patients, which may not fully represent African-American gliomas. Indeed, one of the recurring issues in studying race-based differences in cancer is that African-Americans are less likely than Whites to volunteer for research projects like TCGA [33,34]. Future work will therefore be needed to extend these observations in the study with larger sample size, including increased efforts to obtain participation by African-Americans in biospecimen and outcomes-based research.

In conclusion, we found that African American GBM patients actually tend to survive longer than White GBM patients, and that this may be influenced by initial KPS score and differing tumor genotypes, specifically with genes involved in RA metabolism and signaling. The future exploration of these genes and pathways should provide a more detailed understanding of race-based differences in glioma biology, and may inform novel therapies for this incurable disease.

Conclusions

African Americans have prolonged survival with glioma which is influenced only by initial KPS score. Genes previously associated with both racial disparities in cancer and pathways associated with RA metabolism may play an important role in glioma etiology. In the future exploration of these genes and pathways may inform novel therapies for this incurable disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Results of Cox regression for Glioma and GBM patients with KPS 80.

Hazard ratios and 95%CI were calculated using Cox proportional hazards model with restricted cubic spline method for all glioma patients (A) and GBM patients with KPS 80 (D). (B) and (C), the predicted survival probability for all glioma patients along the factor of age in the two races, for one year and two years, respectively. (E) and (F), the predicted survival probability for GBM patients with KPS 80 along the factor of age in the two races, for one year and two years, respectively.

Fig. 2. GBM patients with KPS ≥ 80 from TCGA included used downstream gene expression analysis and study schematic from clinical perspective.

(A) Survival comparison between African-Americans and Whites when KPS ≥ 80 is shown, with P -value calculated by log-rank test. (B) Differentially expressed genes between two races are shown, with fold change calculated by mean expression of African-Americans divided by that in Whites. (C) Study schematic on the analyses conducted from clinical perspective.

Table 1

Clinical characteristics of all glioma patients from TCGA included in the analysis

Variable	White N=929	African-American N=64	P value
Age (years)			
N(Missing)	929(0)	64(0)	0.6400
Mean(SD)	51.23±15.90	52.36±14.82	
Median(Q1,Q3)	52.00(38.00–63.00)	51.00(42.00–63.50)	
Minimum, Maximum	10.00–89.00	17.00–79.00	
Gender			
MALE	546(58.77)	35(54.69)	0.5157
FEMALE	383(41.23)	29(45.31)	.
Karnofsky performance score			
<80	127(13.67)	16(25.00)	0.0089
>=80	506(54.47)	25(39.06)	.
Missing	296(31.86)	23(35.94)	.
Histological type			
Astrocytoma	157(16.90)	7(10.94)	0.0065
Oligoastrocytoma	110(11.84)	3(4.69)	.
Oligodendroglioma	159(17.12)	5(7.81)	.
Glioblastoma	503(54.14)	49(76.56)	.
G-CIMP status			
No	506(54.47)	32(50.00)	0.0933
Yes	389(41.87)	14(21.88)	.
Missing	34(3.66)	18(28.13)	.
Person neoplasm cancer status			
Tumor free	202(21.74)	8(12.50)	0.0809
With tumor	657(70.72)	53(82.81)	.
Missing	70(7.53)	3(4.69)	.
History of neoadjuvant treatment			
No	908(97.74)	62(96.88)	1.0000
Yes	21(2.26)	1(1.56)	.
Missing	0(0.00)	1(1.56)	.
Targeted molecular therapy			
No	548(58.99)	30(46.88)	0.8707
Yes	285(30.68)	14(21.88)	.
Missing	96(10.33)	20(31.25)	.
Radiation therapy			
No	217(23.36)	8(12.50)	0.0586
Yes	667(71.80)	51(79.69)	.
Missing	45(4.84)	5(7.81)	.
Ethnicity			
Hispanic or Latino	24(2.58)	1(1.56)	1.0000

Variable	White N=929	African-American N=64	P value
Not Hispanic or Latino	820(88.27)	54(84.38)	
Missing	85(9.15)	9(14.06)	

This table describes the clinical variables finally included in the analysis of racial disparity on overall survival for all glioma patients from TCGA. G_CIMP status in GBM patients and IDH1 mutation in Grade II & III patients was combined into one common variable: G_CIMP status (See Supplementary method). SD represents standard deviation.

Table 2

Differentially expressed genes between African-American and White GBM patients with KPS 80

Obs	Gene Symbol	Probe Set ID	Gene Title	Fold Change*	Nominal <i>P</i> value	FDR
1	PRAME	204086_at	preferentially expressed antigen in melanoma	2.77	<.0001	0.0654
2	CRYBB2	206778_at	crystallin, beta B2	2.28	<.0001	<.0001
3	CYP26B1	219825_at	cytochrome P450, family 26, subfamily B, polypeptide 1	2.13	<.0001	0.0144
4	P2RX5	210448_s_at	purinergic receptor P2X, ligand-gated ion channel, 5	1.97	<.0001	0.0406
5	DKK2	219908_at	dickkopf WNT signaling pathway inhibitor 2	1.91	<.0001	0.0207
6	NPY5R	207400_at	neuropeptide Y receptor Y5	1.87	<.0001	0.0034
7	TEX14	221035_s_at	testis expressed 14	1.83	<.0001	0.0007
8	PTHR1	205911_at	parathyroid hormone 1 receptor	1.78	<.0001	0.0144
9	CAPN6	217387_at	calpain 6	1.74	<.0001	0.0028
10	PTGFR	207177_at	prostaglandin F receptor (FP)	1.64	<.0001	0.0034
11	KCNMB3	221125_s_at	potassium large conductance calcium-activated channel, subfamily M beta member 3	1.59	<.0001	0.0109
12	KERA	220504_at	keratocan	1.54	<.0001	0.0668
13	OCA2	206498_at	oculocutaneous albinism II	1.52	<.0001	0.0357

* Fold change was defined as mean mRNA expression for African-American GBM patients divided by White patients.

Table 3

Enrichment analysis of the 13 genes

Pathway	Name	P-value	Adjusted p-value	Z-score	Combined score
KEGG 2016	Neuroactive ligand-receptor interaction Homo sapiens hsa04080	0.0007	0.006	-1.89	9.65
	Calcium signaling pathway Homo sapiens hsa04020	0.0059	0.0265	-1.93	7.02
GO biological process 2015	male meiosis (GO:0007140)	0.00008944	0.02370	-2.93	10.96
	negative regulation of retinoic acid receptor signaling pathway (GO:0048387)	0.0002162	0.02518	-2.64	9.73
	regulation of retinoic acid receptor signaling pathway (GO:0048385)	0.0002851	0.02518	-2.62	9.64